EXTENDED REPORT

Radiological outcome in rheumatoid arthritis is predicted by presence of antibodies against cyclic citrullinated peptide before and at disease onset, and by IgA-RF at disease onset

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Objective: To evaluate the significance of antibodies against cyclic citrullinated peptide (anti-CCP) and rheumatoid factors (RFs), before the onset of rheumatoid arthritis and when presenting as early disease (baseline), for disease activity and progression.

Methods: 93 of a cohort of 138 patients with early rheumatoid arthritis (<12 months of symptoms) had donated blood before symptoms of rheumatoid arthritis (defined as pre-patients) and were identified from among blood donors within the Medical Biobank of northern Sweden. Disease activity (erythrocyte sedimentation rate (ESR), C reactive protein, joint score, global visual analogue scale) and radiological destruction in hands and feet (Larsen score) were assessed at baseline and after two years. Anti-CCP antibodies and RFs were analysed using enzyme immunoassays. HLA shared epitope (SE) alleles (DRB1*0401/0404) were identified.

Results: Patients with anti-CCP antibodies before disease onset had significantly higher Larsen score at baseline and after two years. In multiple regression analyses baseline values of anti-CCP/IgA-RF/IgG-RF/IgM-RF, swollen joint count, and Larsen score significantly predicted radiological outcome at two years. In logistic regression analyses, baseline values of anti-CCP antibodies/IgA-RF, therapeutic response at six months, and swollen joint count/ESR significantly predicted radiological progression after two years. The baseline titre of anti-CCP antibodies was higher in patients with radiological progression and decreased significantly in those with response to therapy. SE allele carriage was associated with a positive test for anti-CCP antibodies in pre-patients and in early rheumatoid arthritis.

Conclusions: Presence of anti-CCP antibodies before disease onset is associated with more severe radiological damage. The titre of anti-CCP antibodies is related to disease severity.

Detection of antibodies against cyclic citrullinated peptides (CCP) has developed during recent years as a valuable tool in diagnosing rheumatoid arthritis and predicting the clinical outcome.[1–3] We have previously shown that anti-CCP antibodies and rheumatoid factors (RFs) of all isotypes predated the onset of rheumatoid arthritis by several years. The presence of anti-CCP antibodies and IgA-RF predicted the development of rheumatoid arthritis, with anti-CCP antibodies having the highest predictive value.[4] Anti-CCP antibodies have also been shown to predict disease activity. Persistent arthritis after two years was strongly predicted by a positive test for anti-CCP antibodies[5] and patients with anti-CCP antibodies had a significantly larger number of joints involved after three years.[6] In patients with undifferentiated arthritis the presence of anti-CCP antibodies predicted the development of rheumatoid arthritis after three years of follow up.[7] Several studies have shown that radiological outcome can be predicted by the presence of RF[8 9] and anti-CCP antibodies[10 11] at the time of diagnosis of early rheumatoid arthritis.

An association between certain alleles of the HLA-DRB1 locus, the so called ''shared epitope'' (SE) and rheumatoid arthritis is well described[12 13] and also associated with a more severe disease progression.[14 15] We previously reported that the presence of anti-CCP antibodies together with SE allele carriage is associated with a very high relative risk for the future development of rheumatoid arthritis[16]. An association between anti-CCP antibodies and SE allele carriage has been reported,[16 17] together with a more severe disease progression in patients with both anti-CCP antibodies and SE.[18]

In this study of a cohort of patients with early rheumatoid arthritis we investigated the presence of anti-CCP antibodies and RFs in blood samples collected before the onset of any symptoms of joint disease in individuals who had donated blood samples to the Medical Biobank, and, in the whole cohort, at the time of diagnosis. The significance of the presence of these antibodies before and after disease onset for disease activity and radiological progression and outcome, as well as their relation with SE, was evaluated. The impact of the titre of anti-CCP antibodies was also studied.

METHODS

Subjects

The register of all patients (n = 138; 98 female, 40 male) with early rheumatoid arthritis (duration less than one year) fulfilling the American College of Rheumatology (ACR) classification criteria for rheumatoid arthritis[19] at the Department of Rheumatology, University Hospital, Umeå

Abbreviations: ACR, American College of Rheumatology; anti-CCP, antibodies against cyclic citrullinated peptide; DAS28, 28 joint disease activity score; DMARD, disease modifying antirheumatic drug; EULAR, European League Against Rheumatism; NSHDS, Northern Sweden Health and Disease Study; RF, rheumatoid factor; SE, shared epitope

Table 1 Larsen score at baseline and at two years in patients with early rheumatoid arthritis who were positive or negative for anti-CCP antibodies, IgG-RF, IgA-RF, or IgM-RF before symptoms of joint disease

	Pre-patient antibodies							
	Anti-CCP		IgG-RF		IgA-RF		IgM-RF	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Baseline	8 (1.5)* (n = 25)	5 (0.7) (n = 58)	6 (1.2)^{ns} (n = 10)	4 (0.7) (n = 52)	5 (1.6) ^{ns} (n = 22)	4 (0.8) (n = 40)	7 (1.7)^{ns} (n = 13)	4 (0.7) (n = 49)
2 years	14 (2.3)* (n = 19)	9 (1.2) (n = 49)	10 (2.3)^{ns} (n = 10)	10 (1.3) (n = 52)	12 (2.0)^{ns} (n = 22)	9 (1.4) (n = 40)	14 (3.0)^{ns} (n = 13)	9 (1.2) (n = 49)
p Value†	<0.001	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001	<0.01

Values are mean (SEM).
*p<0.05 (positive compared with negative).
^{ns}Not significant (positive compared with negative).
†Significance of the difference between baseline values compared with scores at two years.
Anti-CCP, antibodies against cyclic citrullinated peptide; neg, negative; pos, positive; RF, rheumatoid factor.

(the only rheumatology department in the county of Västerbotten) and with a known date for the onset of symptoms, was co-analysed with the register of individuals in the Northern Sweden Health and Disease Study (NSHDS) cohort and the maternity cohort of northern Sweden (Medical Biobank), Umeå, Sweden. The NSHDS consists of three subcohorts which all are population based. All adults in the county of Västerbotten are invited to participate and no one is excluded. The subcohorts and the conditions for recruitment into the cohorts and the collection and storage of blood samples have been described in detail previously.[4] Ninety three of the 138 patients with early rheumatoid arthritis (72 female, 21 male) were identified as having donated blood before the onset of any symptoms of joint disease. These 93 individuals are referred to as pre-patients. The median time of blood sampling before the onset of symptoms was 3.0 years (interquartile range (IQR), 1.1 to 5.3). Mean age at onset of symptoms was 54 years (range 23 to 73). The median time from onset of symptoms until the diagnosis of early rheumatoid arthritis (⩾4 ACR criteria fulfilled) was 7.0 months (IQR, 5.0 to 9.0). The patients were followed for two years from the date of diagnosis. Follow up data from the early rheumatoid arthritis clinic were missing for five individuals; two were not examined at the correct time points because they had palindromic rheumatoid arthritis and another was examined only at baseline. Two were followed for only six months because one had died and the other refused to participate further. The patients were treated with the aim of achieving remission using disease modifying antirheumatic drugs (DMARDs), corticosteroids, non-steroidal anti-inflammatory drugs, and analgesics with respect to the clinical situation. During the study, 92% of the patients were treated for at least six months with DMARDs: 68% received methotrexate, 30% sulfasalazine, 14% gold in either oral or parenteral form, 12% antimalarials, 0.7% tumour necrosis factor α blockers, 12% other DMARDs, and 30% combination therapy. Forty eight per cent of the patients were receiving low dose prednisolone (⩽10 mg/day) for at least six months during the study.

The ethics committee of the University Hospital, Umeå, approved the study and the patients gave their written informed consent.

Enzyme immunoassays for anti-CCP-2 antibody

Anti-CCP antibodies were measured in plasma or serum from pre-patients, Biobank samples (n = 93), and at the diagnosis of rheumatoid arthritis (baseline; n = 85) using the Immunoscan rheumatoid arthritis (Mark 2) enzyme immunoassay (EIA) from Euro-Diagnostica (Arnhem, Netherlands) (cut off value 25 units/ml) as previously described.[4] The Diastat kit from Axis-Shield Diagnostics (Dundee, UK) (cut off value 5 units/ml) was used for analyses both at baseline (n = 101) and after two years. Consequently, at baseline anti-CCP antibodies were analysed in a total of 127 patients and in 59 of the samples using both methods, and there was total agreement between the results. The two methods are based on the same type of peptides.[20] All measurements were carried out in duplicate.

Enzyme linked immunosorbent assays for isotype specific RFs

RFs of IgG, IgM and IgA isotypes were measured by in house enzyme linked immunosorbent assays (ELISAs), as previously described.[4] The 95th centile value of the controls, analysed previously, was used as the cut off point for all three RF classes.[4] The analyses were undertaken in pre-patient samples (n = 65) and samples were collected at baseline (n = 126).

HLA-DRB1 genotypes

HLA-DRB1 genotyping was carried out on samples collected at baseline using polymerase chain reaction sequence specific primers from a DR low resolution kit and DRB1*04 subtyping kit (Dynal, Oslo, Norway).[21 22] The SE alleles were defined as HLA DRB1*0401 or DRB1*0404.

Measures of disease activity

The erythrocyte sedimentation rate (ESR, mm/h) and plasma concentrations of C reactive protein (mg/l) were determined at baseline and after 0.5, 1, and 2 years. At the same time

Table 2 Larsen score at two years for the whole early rheumatoid arthritis cohort stratified on different antibodies at baseline

Antibody	Positive	Negative	p Value
Anti-CCP	11.2 (0.9) (n = 83)	7.5 (1.7) (n = 28)	<0.05
IgG-RF	13.4 (1.2) (n = 54)	7.6 (0.9) (n = 58)	<0.0001
IgA-RF	11.2 (1.0) (n = 90)	6.2 (1.0) (n = 22)	<0.001
IgM-RF	10.8 (0.9) (n = 98)	7.2 (1.6) (n = 14)	NS

Values are mean (SEM).
CCP, cyclic citrullinated peptide; RF, rheumatoid factor.

Table 3 Result of four different multiple regression analyses with radiological outcome at 24 months as dependent variable, and as independent variables baseline values of Larsen score, swollen joint count, and one of each: IgA-RF, IgG-RF, IgM-RF, or anti-CCP antibodies, respectively in patients with early rheumatoid arthritis

	Regression analyses							
Variable	**B**	**p Value**	**B**	**p Value**	**B**	**p Value**	**B**	**p Value**
Larsen score	0.97	<0.0001	0.95	<0.0001	0.99	<0.0001	0.97	<0.0001
Swollen joints	0.28	<0.01	0.20	<0.05	0.30	<0.01	0.31	<0.01
IgA-RF	4.0	<0.01	–	–	–	–	–	–
IgG-RF	–	–	2.8	<0.05	–	–	–	–
IgM-RF	–	–	–	–	4.2	<0.05	–	–
Anti-CCP	–	–	–	–	–	–	2.8	<0.05
R^2	50.9%		50%		50%		49.4%	
R^2 adjusted	49.5%		48.6%		48.6%		48.0%	

Anti-CCP, antibodies to cyclic citrullinated peptide; RF, rheumatoid factor.

points, clinical examination including a 28 joint count for tender and swollen joints and assessment with a global visual analogue scale (VAS) (EULAR criteria) were undertaken and the disease activity score including 28 joints (DAS28) was calculated.[23] Response to treatment was determined according to EULAR response criteria.[24]

Measures of disease outcome

Posterior-anterior radiographs of the hands, wrists, and feet were obtained at baseline (n = 127) and after two years (n = 112) and were examined blind by two rheumatologists (EB and SRD) specially trained in the evaluation of *x* rays, and graded according to the Larsen score[25] by comparison with standard reference films. The scoring system included 32 areas: metacarpophalangeal joints II–V (n = 8), all proximal interphalangeal joints (n = 8), the wrists divided into four areas (n = 8), and metatarsophalangeal joints II–V (n = 8). Each joint or joint area was graded from 0 to 5.[25] The maximum score was 160.

Statistics

Differences in continuous data between two groups were analysed using an independent *t* test. Differences in continuous data from two different time points in the same individual were analysed with a paired *t* test. Variations over time between and within groups were assessed by analysis of variance for repeated measurements (StatView, version 4.51; Abacus Concepts, Berkeley, California, USA). The χ^2 test was used for testing categorical data between groups. Multiple regression analyses were carried out using the analysis of variance (ANOVA) general linear model. Factors and covariates were chosen with respect to results of simple regression analyses or clinical assumptions. Backward logistic multivariate regression analyses were used to estimate the odds ratio for radiological progression at two years. Radiological progression was defined as present only if the difference in Larsen score at baseline and two years was greater than the median value. The degree of explanation of variations in the dependent variable given by the independent variables was expressed as Nagelkerke R^2. All p values are two sided, and p values equal to or less than 0.05 were considered statistically significant. All calculations, except analysis over time, were carried out using the SPSS for Windows (version 11.5; Chicago, Illinois, USA).

RESULTS

Among the individuals identified in the early rheumatoid arthritis cohort as donors of blood to the Medical Biobank before the onset of any symptoms of joint disease (median 3.0 years (IQR, 1.1 to 5.3)), anti-CCP antibodies were found in 32.3% of the pre-patient samples (n = 93). The corresponding figures for IgM-RF, IgA-RF, and IgG-RF were 20.0%, 36.9%, and 16.9%, respectively. When the patients were diagnosed as having early rheumatoid arthritis (baseline) (n = 138; median time after onset of symptoms 7.0 months (IQR, 5.0 to 9.0)) the prevalence of all antibodies had increased, with IgM-RF being the most prevalent at 85.7%. The corresponding figures for anti-CCP antibodies, IgA-RF, and IgG-RF were 74.8%, 78.6%, and 46.8%, respectively. The prevalence of SE allele was 54.8% (51/93) in the pre-patient group, and in the early rheumatoid arthritis group (containing 45 additional individuals, with no pre-patient samples) it was 58.7% (81/138). There was a significant association between carriage of the SE allele and a positive test for anti-

Table 4 Predictors of radiological progression at two years in patients with early rheumatoid arthritis

	Model 1*		Model 2†	
Variable	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value**
Anti-CCP	5.4 (1.7 to 17.0)	<0.01		
Swollen joints	1.1 (1.0 to 1.2)	<0.05		
Therapeutic response‡	0.35 (0.14 to 0.85)	<0.05	0.41 (0.17 to 0.97)	<0.05
IgA-RF			9.8 (2.1 to 45.5)	<0.01
ESR			1.02 (1.00 to 1.04)	0.05

Variables remaining significant after backward stepwise logistic regression analyses including baseline values of anti-CCP antibodies/IgA-RF (yes/no), swollen joint count, ESR, Larsen score, shared epitope (yes/no), and therapeutic response at six months (yes/no).
*The model included anti-CCP antibodies (yes/no), but not IgA-RF.
†The model included IgA-RF (yes/no), but not anti-CCP antibodies.
‡EULAR response criteria, no *v* good/moderate response.
Anti-CCP, antibodies to cyclic citrullinated peptide; CI, confidence interval; ESR, erythrocyte sedimentation rate; OR, odds ratio; RF, rheumatoid factor.

CCP antibodies either in the pre-patient group ($\chi^2 = 4.1$, $p<0.05$) or in the early rheumatoid arthritis group ($\chi^2 = 6.0$, $p<0.05$). SE allele carriage was also significantly associated with IgM-RF in the early rheumatoid arthritis group ($\chi^2 = 4.0$, $p<0.05$), but not in the pre-patient group. No significant associations were found between SE and IgG-RF or IgA-RF (data not shown).

Relation between clinical data and anti-CCP antibodies and RFs in pre-patients

The Larsen score at baseline was significantly higher ($p<0.05$) in patients with anti-CCP antibodies predating symptoms than in those without (table 1). Additionally, two years after diagnosis a significantly higher Larsen score was found in patients who were seropositive for anti-CCP antibodies before disease onset. The Larsen score increased significantly from baseline to two years, both in patients positive and negative for predating anti-CCP antibodies (table 1). Patients positive for both predating anti-CCP antibodies and SE (n = 15) had a slightly greater radiological progression (see Statistics for definition) from baseline to two years, and a higher Larsen score at baseline and at two years than patients without SE and without pre-dating anti-CCP antibodies (n = 26), although the difference was not statistically significant (data not shown).

There was no significant difference in the Larsen score at baseline or at two years in RF positive individuals compared with those without predating RFs of any isotype (table 1), nor was there any relation between either predating anti-CCP antibodies or RF isotypes and measures of inflammation such as DAS28, ESR, C reactive protein, and tender or swollen joint count at baseline or at two years (data not shown).

Relation between anti-CCP antibodies and RFs at disease onset, and clinical data

Patients positive for anti-CCP antibodies, IgG-RF, and IgA-RF at baseline had a significantly worse Larsen score at two years than patients without these antibodies (table 2). At baseline the Larsen score was significantly higher in patients positive for IgG-RF than in those negative for IgG-RF, but no differences were detected between patients positive or negative for anti-CCP antibodies, IgA-RF, or IgM-RF (data not shown).

In multiple regression analyses, the baseline values of swollen joint count, Larsen score, and anti-CCP antibodies/IgA-RF/IgG-RF/IgM-RF significantly predicted a greater Larsen score at two years (table 3).

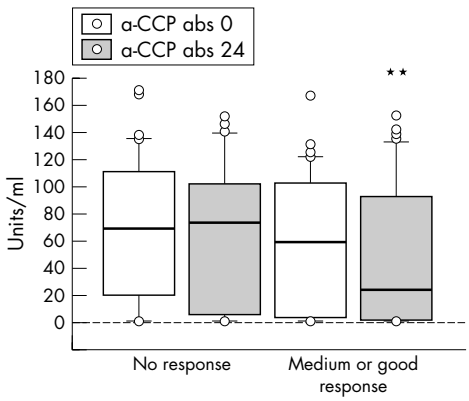

Figure 1 Titre of anti-CCP antibodies (units/ml; Diastat ELISA; median values, 25th and 75th centiles, and range) at baseline (a-CCP abs 0) and at two years (a-CCP abs 24), stratified for different groups of response to treatment at six months (EULAR response criteria). No response, n = 43; median or good response, n = 57. **p<0.01. a-CCP abs, anti-cyclic citrullinated peptide antibodies; ELISA, enzyme linked immunosorbent assay; EULAR, European League Against Rheumatism.

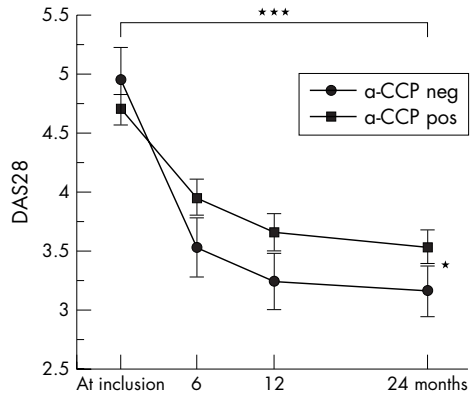

Figure 2 Disease activity expressed as DAS28 at different time points after diagnosis of early rheumatoid arthritis in patients positive (a-CCP pos; n = 95) or negative (a-CCP neg; n = 32) for anti-CCP antibodies at baseline. Values are means, error bars = SEM. *p<0.05; ***p<0.001. a-CCP, anti-cyclic citrullinated peptide antibodies; DAS28, 28 joint disease activity score; neg, negative; pos, positive.

Anti-CCP antibodies and RFs were not included in the same model because they were significantly associated ($\chi^2 = 38.0$, $p<0.0001$ for anti-CCP and IgA-RF; $\chi^2 = 4.4$, $p<0.05$ for anti-CCP and IgG-RF; and $\chi^2 = 22.9$, $p<0.0001$ for anti-CCP and IgM-RF). In a backward stepwise logistic regression analysis to identify predictors for radiological progression at two years, baseline values for anti-CCP antibodies/IgA-RF/IgG-RF/IgM-RF (yes/no), ESR, swollen joint count, Larsen score, SE (yes/no), and therapeutic response at six months (yes/no) were included. Anti-CCP antibodies and swollen joint count at baseline and therapeutic response at six months significantly predicted radiological progression at two years, explaining about 21% of the variation (Nagelkerke R^2) and allowing correct classification in 73% of cases (table 4). In the model with IgA-RF, ESR at baseline and therapeutic response at six months significantly predicted radiological progression at two years (table 4). Nagelkerke R^2 was 24% and the accuracy 67%. In the model with IgG-RF, therapeutic response apart from IgG-RF significantly predicted radiological progression, however with lower Nagelkerke R^2 (14%) and accuracy (64%; data not shown). No significant prediction of radiological progression was found when IgM-RF was in the model.

Patients with both SE allele and anti-CCP antibodies at baseline (n = 55) had a higher Larsen score at two years than patients negative for both factors (n = 17), at 12 (1.1) *v* 6 (1.3), $p<0.01$ (mean (SEM)). The radiological progression from baseline was also higher in patients with anti-CCP antibodies and SE than in those negative for both factors, at 6.0 (0.79) *v* 2.0 (0.42), $p<0.001$. The Larsen score at baseline did not, however, differ significantly between positive results for baseline anti-CCP antibodies and SE (6 (0.65)) and negative results for both factors (4 (1.1)).

The titre of anti-CCP antibodies decreased significantly between baseline and two years in those patients who had medium or good response to therapy at six months (mean, 59 to 49 units/ml; $p<0.01$; n = 57) (fig 1), at 12 months (mean, 60 to 53 units/ml; $p<0.05$; n = 65), and at two years (mean, 62 to 54 units/ml; $p<0.01$; n = 71), respectively. Patients with radiological progression had a higher titre of anti-CCP antibodies (Diastat ELISA) at baseline than those without progression, at 73 (7.5) *v* 51 (6.4) units/ml (mean (SEM)), $p<0.05$.

In the patients with early rheumatoid arthritis there was a significant reduction of DAS28 over time ($p<0.0001$); however, the reduction was less in patients positive for anti-CCP antibodies at baseline ($p = 0.05$; fig 2). Patients

positive for IgM-RF at baseline also had less reduction in DAS28 over time compared with IgM-RF negative patients ($p<0.01$; data not shown). The other RFs did not have any impact on DAS28 over time (data not shown).

DISCUSSION

This is the first study in which the significance of the presence of anti-CCP antibodies before disease onset on the radiological outcome in rheumatoid arthritis has been investigated. Individuals positive for anti-CCP antibodies before the onset of symptoms of joint disease had significantly more joint erosions at the time of diagnosis of rheumatoid arthritis than individuals negative for these antibodies. Furthermore, two years after diagnosis the radiological outcome was worse for those seropositive for anti-CCP antibodies before the diagnosis of rheumatoid arthritis. The anti-CCP antibodies predated the onset of signs and symptoms of joint disease by up to several years. These observations suggest a subclinical process related to the presence of anti-CCP antibodies, whereby the formation of erosions has already started without a clinically evident inflammatory process. At their first visit to the early rheumatoid arthritis clinic, patients positive for anti-CCP antibodies as pre-patients or at baseline, or both, did not have a higher level of inflammatory activity than antibody negative patients. However, the measurements of the inflammatory activity (ESR, C reactive protein, joint count) may be considered relatively rough. In contrast to the present study, Kastbom *et al*[26] were able to demonstrate significantly higher baseline ESR and C reactive protein in anti-CCP antibody positive than antibody negative patients when they presented at the early arthritis clinic.

In the present study, RFs of all isotypes were also present in pre-patient samples, but no significant differences in the Larsen scores at baseline or at two years were detected between individuals with pre-dating RFs of any isotype and those without. One interpretation of this finding is that presence of anti-CCP antibodies in pre-patient samples predicts a more aggressive form of rheumatoid arthritis. However, for diagnostic sensitivity the cut off level for positive RFs was at the 95th centile. To analyse the severity of the disease, perhaps higher cut off levels for RFs with a reduced sensitivity would be more appropriate. This should be further investigated in another study. Another point to take into consideration is that the rather small number of individuals in the analyses of RFs decreases the possibility of obtaining statistically significant differences.

The results presented here, that the presence of anti-CCP antibodies at disease onset is associated with radiological progression, confirm earlier reports.[11 27] The finding[11] that radiological progression was also predicted by initial radiological joint damage was not, however, confirmed in our study. We found that radiological damage at two years, but not radiological progression, was predicted by baseline radiological damage. One interpretation of this discrepancy might be that our patients had a better response to pharmacological therapy, which slowed the joint damaging process. Using multiple regression analyses in the present study showed that baseline values of swollen joint count, Larsen score, and anti-CCP antibodies or RFs of each isotype independently predicted the higher Larsen score at two years and accounted for approximately half the variation. There was a correlation between anti-CCP antibodies and RFs in baseline samples, which explains the similar results. However, the results of logistic regression analyses favour anti-CCP antibodies and IgA-RF as the most important predictors, which is consistent with the results of Lindqvist *et al*.[27] In several previous studies an association between initial inflammation, measured as ESR, C reactive protein, or number of swollen joints, and radiological outcome or progression has been reported.[5 10 11 27 28] In multiple regression analyses,[10 27] however, anti-CCP antibodies and RFs (IgM-RF and IgA-RF) have been better predictors for radiological outcome and progression than measures of inflammation, which is in agreement with our results.

Patients with anti-CCP antibodies or IgM-RF at baseline had a significantly smaller reduction in the DAS28 during the follow up period than the antibody negative patients, indicating more persistent disease activity in patients with these antibodies. These results are similar to those reported by others.[5 11 26] Our aim in this study was to investigate the significance of anti-CCP antibodies and RFs for disease activity and severity. As anti-CCP antibodies and RFs are strongly correlated, comparisons between them as predictors are difficult; however, the results of this study favour the view that IgA-RF in addition to anti-CCP antibodies could be related to radiological progression, while both IgM-RF and anti-CCP antibodies could be related to inflammatory activity, as measured by DAS28, and to SE allele.

High titres of anti-CCP antibodies at baseline were related to greater radiological progression at two years. The titres declined during the study in those patients with a therapeutic response. This is in agreement with the results of other studies showing significant decrease in anti-CCP antibody titres in patients with early rheumatoid arthritis who had a decrease in disease activity.[5 6 26] In our study the therapeutic response predicted less radiological progression after two years, in contrast to the presence of anti-CCP antibodies or IgA-RF at baseline, which predicted increased radiological progression. This suggests that repeated measurements of the anti-CCP antibody titre could be of clinical use for assessing disease activity and severity. In contrast to our findings, Mikuls *et al*[29] reported that disease duration but not treatment response to conventional DMARDs was associated with declines in the anti-CCP-antibody levels. Unfortunately corresponding data for the course of the titres of RFs are missing in the present study.

There was a significant association between carriage of SE allele and a positive test for anti-CCP antibodies in both the pre-patient samples and in samples collected at baseline, which is consistent with our earlier results[14] and those of others.[11 17] Hill *et al*[30] showed that conversion of arginine to citrulline in HLA-DRB1*0401 transgenic mice significantly increased peptide-MHC affinity and led to activation of CD4+ T cells. This finding may explain B cell activation and the production of anti-CCP antibodies found preferentially in individuals carrying SE allele. Patients with both the SE allele and anti-CCP antibodies at baseline showed a more severe radiological outcome than those lacking both. This concurs with the study by van Gaalen *et al*,[18] who reported an association between HLA class II rheumatoid arthritis susceptibility alleles and the production of anti-CCP antibodies as well as an increased rate of joint destruction in patients with both SE allele and anti-CCP antibodies. In an earlier study,[16] we found that the combination of SE allele and anti-CCP antibodies in individuals without symptoms of joint disease was associated with a very high relative risk for the future development of rheumatoid arthritis. The results of the present study suggest that the combination of SE allele and anti-CCP antibodies is also associated with disease severity.

Conclusions

The presence of anti-CCP antibodies before and at disease onset is associated with a more aggressive disease as measured by a significantly higher Larsen score. Anti-CCP antibodies together with SE allele carriage are markers for severe disease outcome. The titres of anti-CCP antibodies decreased significantly in patients with therapeutic response,

suggesting that they could be a useful marker of disease progression. Anti-CCP antibodies or any of the RFs, swollen joint count, and Larsen score at baseline significantly predicted radiological outcome at two years. Radiological progression was predicted by both anti-CCP antibodies and IgA-RF, in combination with clinical data.

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